



Principal component analysis (PCA) on temporal changes of soil health indicators

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Article Info

Received : 5th June 2023

Revised : 14th July 2023

Accepted: 21th July 2023

Keywords:

agriculture management practices,
cropping systems, soil quality,
total organic carbon (TOC)

Abstract

Soil health indicators are related to environmental factors, such as nutrient management, crop practices, different cropping systems, and biodiversity. 14 soil health indicators were measured and compared in our study to clarify the impact of different cropping system on soil quality. The primary comparisons were carried out among perennial plants, annual plants, and cover cropping, with an examination of the tillage system and fertilization taken into account during the analysis. Multivariate analysis recorded that the most promising indicators of soil health that related to soil quality and health were organic carbon (OC), total carbon (TC), followed by fall soil total nitrogen (TN). The main factor for clustering treatments based on indicators was N fertilization according to Euclidean distance that was applied to measure similarity of the groups. Although perennials and cover crops recorded more carbon sequestration and TC improvement, corn crops showed the worse impact on soil pH and bulk density (BD). Non-tillage practices significantly increased wet aggregate and soil moisture. The combination of TC field measurements with long-term cropping systems information has improved our understanding of how different cropping practices influence SOC improvement in soil full profile. It would develop appropriate and cost-effective agricultural management activities, maintain or improve carbon soil accumulation to guide farmer decision making, and ultimately advance food and nutritional security.

INTRODUCTION

The significant role of soil health indicators raises when sustainable agriculture faces the problem of massive production, specially under climate change and food demand. Environmental changes and stressors also greatly impact soil quality. For example, salt stress drops soil health, limits plant growth/ yield, causes land degradation, and negatively impacts on the total amount of chlorophyll and carotenoids (Mirbakhsh and Sedeh, 2022). Gene expression alters under different soil EC and causes lower quality of the production (Mitrano et al., 2012). Therefore, to meet demands for food and global changes, some strategies, such as uncontrolled rate of manure and nano-fertilizer, were applied, which put water quality and soil health

in danger. For example, an unbalanced presence of nano-particles alters the process of photosynthesis and initiates oxidative stress through the production of reactive oxygen species (ROS). This leads to DNA degradation, cell death, and a decrease in antioxidant enzyme levels. Furthermore, these nano-particles and micro pollutants are transported as remnants, flowing through rivers or lakes and posing a significant risk to human and animal health (Mirbakhsh, 2023).

In this situation, soil can play a key role to protect health and reduce environmental contamination. Soil health indicators are responsive to cropping system under the stress-based ecosystems to promote sufficient food to combat with malnutrition and hunger (Augustine and Lane, 2014). By focusing on measuring soil health indicators, a more profound

understanding of soil properties can be obtained. It is crucial to accurately and effectively measure the biological, chemical, and physical aspects of the soil considering the diverse range of soil types and climate conditions (Franzluebbers, 2016).

Ashworth et al. (2018) revealed that management practices had great impact on soil characteristics. Sustainable agricultural practices can contribute to the preservation and improvement of soil health, thereby ensuring the long-term sustainability of soil resources (Norris et al., 2020). The choice of suitable cropping practices for increasing soil organic matter content should focus on several factors such as GHG emissions mitigation, carbon capture improvement, and temporal yield stability (Knapp and van der Heijden, 2018; Somerville et al., 2010). For example, well-managed arable land conversion to perennial has been resulted in accumulation of SOC due to their low nutrient requirements, extensive fibrous root systems, and subsequent turnover of aboveground biomass (Cadoux et al., 2014; Ledo et al., 2020; Chen et al., 2022) or no-tillage reduced soil pH, but raised organic matter and Mg, Ca, and K concentrations compared to conventional tillage (Tarkalson et al., 2006).

Long-term monitoring on crop type management and former land-use history is needed to accurately quantify carbon sequestration potential on these pastures. In order to mitigate climate change and enhance soil health, a key strategy is to focus on maximizing the stabilization and accumulation of soil carbon (C) in deeper layers of the soil. This approach plays a central role in successful efforts to minimize soil organic carbon (SOC) loss in agricultural lands. By prioritizing the preservation of SOC, we can contribute to climate change mitigation and improve

the overall health of the soil (Chen et al., 2022; Sun et al., 2021; Crow et al., 2018; Lal et al., 2013).

The objective of this study was to investigate the impact of various cropping systems and management. Combining annual crops and cover crops was expected to result in better soil factors and greater potential for crop production compared to the normal practice of annual crop-fallow with tillage.

MATERIALS AND METHODS

Field experiment

The research experiment was conducted at the Research Farm of Islamic Azad University, Chalus Branch, located at coordinates of 40° 55' N and 53° 72' E. The farm is situated at an altitude of 4 meters above sea level. The study was carried out during the crop year of 2019–2020. Soil samples were collected at a depth of 0.15 meters in mid-April 2019, before any tillage, planting, or fertilization activities took place. Measurements were taken using intact and repacked soil cores. The relative humidity, mean temperature, and rainfall levels at the study site are provided in Table 1.

The experimental design consisted of 12 treatments arranged in a completely randomized block design with four replications. This design has been implemented since 1995. Among the treatments, there were three options for perennials, including prairie, miscanthus, and switchgrass, which were grown without tillage and without any fertilizer application.

For the annual crops, three treatments were selected. This included corn (CC) and a rotation of corn/soybean-soybean/corn (CS-SC) with tillage. Nitrogen sources for the corn treatments included

Table 1. Meteorological parameters for the field sites during experiment (Mazandaran province Meteorological Office)

Months	Average temperature (°C)		Relative humidity (%)		Precipitation (mm)	
	2019	2020	2019	2020	2019	2020
November	12.98	14.06	79.87	84.32	148.36	193.12
December	10.52	12.13	85.10	79.80	185.30	122.90
January	8.90	10.60	80.30	82.40	95.20	94.30
February	10.30	12.20	84.70	88.20	87.90	94.30
March	8.10	11.20	83.40	86.50	110.10	118.40
April	11.40	14.15	83.10	79.90	70.30	125.10
May	16.40	18.90	80.70	82.10	18.10	41.20
June	24.20	21.50	73.20	79.30	10.30	26.30

urea-ammonium nitrate 28% (w/w) N (UAN) side-dressed at the V5 growth stage of corn. The application rates were 136 kg N ha⁻¹ yr⁻¹ for continuous corn and 156 kg N ha⁻¹ yr⁻¹ for corn soybean in rotation. Liquid swine manure, with a C/N ratio of 2:1 and 80% (w/w) of N as NH⁴⁺, was applied at a rate of 257 ± 25 kg N ha⁻¹ yr⁻¹ in either the spring (SM) or the fall (FM) for continuous corn. Cover cropping with cereal rye (*Secale cereale*) and white winter clover (*Trifolium repens*) was added to sorghum and corn in different plots. In fall, chisel tillage was performed, and in spring, chisel plus disk tillage was conducted in all cropped plots except for the perennial treatments. The planting density was 142 and 490 seeds ha⁻¹ for the respective crops. Soil tests were conducted each fall to assess general fertility using established protocols and pathways. The results indicated that soil pH, potassium (K), and phosphorus (P) levels were not limiting factors in the experiment.

Soil sampling was carried out during corn planting, specifically in early June, on two separate occasions: June 11, 2019, and June 3, 2020. Additionally, soil sampling was conducted at the R1 stage of maize growth, which occurred in late July, either on July 25, 2019, or July 27, 2020.

Soil sampling and analysis

A specific portion of the soil, measuring 4 cm wide by 1.5 cm thick by 15.5 cm deep, was selected from three sides of the hollow using a cutter. These soil samples were collected and placed in a bag to create a representative sample for further study. The bag containing the soil samples was then stored in a cooler packed with ice to maintain their freshness during transportation back to the laboratory. For the analysis of biological properties, a subsample weighing 400 grams from each plot was sealed in a plastic bag. These samples were also placed in a cooler packed with ice and shipped to soil testing laboratories. To determine the total carbon (C) and nitrogen (N) concentration in the soil, the samples were first sieved through a 2-mm sieve. Then, a 0.5 gram sub-sample from each core was combusted using a CN analyzer to measure the C:N ratio. To improve the distribution of the soil C data, a square root transformation was applied prior to analysis. The organic matter (OM) content of the soil is directly influenced by bulk density. Therefore, accurate measurement of bulk density is crucial for converting

soil organic carbon (SOC) on a weight basis to content per unit volume. The soil cores were oven dried to obtain their dry mass, which was then used to calculate bulk density. Bulk density was determined by dividing the dry mass of the soil samples by the volume of the soil core (Grossman and Reinsch, 2002). To ensure representative sampling throughout the soil profile, samples were collected from the center of each depth interval, allowing for accurate characterization of each depth increment. The process of soil aggregation is multifaceted, involving intricate interactions between plant roots and mycorrhizal fungi, along with the composition of the plant community. The presence of cover crops and the release of fresh root exudates can enhance the aggregation process, thereby influencing the sequestration of soil organic carbon (SOC). These factors play a significant role in improving the overall health and stability of the soil (Lal, 2015).

In this phase of the project, soil samples were collected to measure the wet aggregate stability (WAS). A hydraulic probe with a diameter of 5.3 cm was used to obtain samples from the four specified depths. These samples were then pushed through an 8 mm sieve while still moist. Afterward, they were dried and sieved again to remove the fraction larger than 2 mm. From each sample, two 25 g subsamples were derived for analysis using the wet aggregate size distribution method. Wet aggregates were calculated by utilizing a sprinkle infiltrometer. This involved measuring aggregates in the 0.25–2.00 mm size range that remained on a 0.25 mm sieve after a simulated hard rainfall lasting for 5 minutes (Schindelbeck et al., 2016). The average mean weight diameter will be calculated for each depth. Soil pH and EC were calculated using 1:2 soil/water ratio with a pH meter (Thomas, 1996). To determine the concentrations of soil phosphorus (P), calcium (Ca), nitrogen (N), and potassium (K), the soil samples were extracted using the Mehlich 3 solution. This extraction method allows the release of these nutrients from the soil. The extracted solution was then analyzed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) at the Plant & Innovation Lab in Sari, Mazandaran. ICP-AES is a technique that utilizes plasma and atomic emission to accurately quantify the concentrations of various elements in the sample (Sikora and Moore, 2014).

Data analysis

The data from the study, including all the different treatments, were analyzed using R version 4.0, developed by the R Core Team in 2020. To perform a cluster analysis, the first step involved implementing principal component analysis (PCA). PCA, as described by Jolliffe in 1986, was used to reduce the dimensionality of the dataset by projecting it onto a lower-dimensional space. This technique helps identify and capture the most significant patterns and variations in the data (Rossel et al., 2016).

In this study, the relationships between soil factors and treatments were examined using principal component analysis (PCA) with the vegan package (available at <https://github.com/vegandevs/vegan>).

The aim was to reduce the number of correlated soil health indicators by selecting the most significant variables. The data were analyzed using PCA, which allowed the identification of the most differentiating soil health indicators correlated with mean annualized crop yield over the years. To assess the common soil health indicators among different treatments, hierarchical clustering with Ward's algorithm was performed using the PCA results. This clustering method helps identify treatments that exhibit similar variations in concentrations of the soil health indicators. Furthermore, a correlation matrix was analyzed to examine the relationships between the treatments. This matrix provided insights into how the treatments relate to each other based on the concentrations of the soil health indicators.

Table 2. Soil chemical and physical properties by comparing different cropping system and tillage practices

Treatments	Tillage	Moi	PH	BD	WAS	Nmin	Ca ⁺²	K ⁺	Mg ²⁺	P	TN	TC	OC	Cmin
Prairie (Trt1) Perennial	N	24.7	6.17	1.311	63.16	48.09	2999.8	178.8	717.4	20.9	2258.7	27692	27692	0.431
Miscanthus (Trt2) Perennial	N	22.45	6.72	1.14	44.95	44.64	3213.8	151.4	782	9.8	2283.13	27725	27725	0.547
Corn+ Cereal Rye (Trt3) Cover-crop	N	20.69	6.58	1.25	43.69	43.69	2948.8	109.2	762.6	6.4	1738.24	18461	18461	0.249
Switchgrass (Trt4) Perennial	N	23.23	5.95	1.39	67.69	67.69	3253.8	165.4	682.8	19.1	2629.64	30658	30658	0.463
White Clover (Trt5) Cover-crop	N	19.79	6.97	1.4	51.55	51.55	2808.8	125.1	709.3	14.9	1992.52	21192	20192	0.241
Soy/Corn (Trt6) +N fertilizer Annual	Y	19.91	6.07	1.32	33.09	33.09	2507.8	108.7	625.5	16.9	1895.03	20138	20138	0.207
Corn/Soy (Trt7) + N fertilizer Annual	Y	22.04	5.76	1.8	45.57	43.17	3018.8	116.4	706.1	16.8	2344.81	26926	26926	0.268
Soy/Corn + Cereal Rye (Trt8) Cover-crop	Y	21.29	6.72	1.9	59.43	41.43	3132.8	129.8	799.3	9.6	1954.69	19958	19958	0.222
Corn/Soy + Cereal Rye (Trt9) Cover-crop	Y	22.33	5.05	1.89	39.97	52.1	2950.8	186.1	654.4	83.2	2553.93	28980	28980	0.244
Corn/Soy + 0 N fertilizer (Trt10) Annual	Y	21.04	6.04	1.81	84.04	42.7	2619.8	117.1	642.1	20.4	2086.74	22362	22365	0.244
Soy/Corn + 0 N fertilizer (Trt11) Annual	Y	24.33	5.73	1.6	32.34	39.51	3261.8	144.7	651.3	20.1	2495.23	30028	30028	0.468
Continuous Corn (Trt12)	Y	19.79	6.89	1.5	43.99	48.34	3277.8	197.1	800.2	67	1861.71	20192	19292	0.339

RESULTS AND DISCUSSION

According to the results, the treatments significantly affected soil moisture (Moi), bulk density (BD), soil total carbon (TC), soil total N (TN), and organic carbon (OC) (Table 2).

Wet aggregate stability (WAS) and soil moisture were greater under no tillage and non-annual system. Root exudates are known as controller for aggregation stability in the rhizosphere (Reid et al., 1982; Helal and Sauerbeck, 1986), which might be the main reason of higher soil moisture and WAS in cover cropping (treatments 3–5) and perennials (Treatments 1–2–4). Cover crop treatment also affected aggregation by providing Glomalin as a beneficial cementing agent for formation, stabilization of aggregate and enhancement of SOC pool, which was produced by arbuscular mycorrhizal fungi (AMF) (Fernandez-Ugalde et al. 2011). Moreover, cover cropping promoted soil

structure and increases the mean residence time of carbon in the soil by increasing CO₂ levels through AFM activity (Lal, 2004). TC was higher in perennial than annual, indicating the influence of cropping practices in soil organic carbon (SOC) and carbon reservoir (Mirbakhsh et al., 2022). The lowest bulk density was recorded in treatment 2 (Miscanthus), showing the impact of this plant on soil health and quality.

The PCA analysis focused on exploring the connections between potential soil health indicators. Among the 12 soil properties examined, carbon was identified as the most promising indicator. Following carbon, the soil total nitrogen, soil calcium (Ca), moisture, and pH were ranked as the next most significant indicators in sequential order (Figure 1). These findings highlight the importance of these indicators in assessing soil health and understanding the relationships between different soil properties.

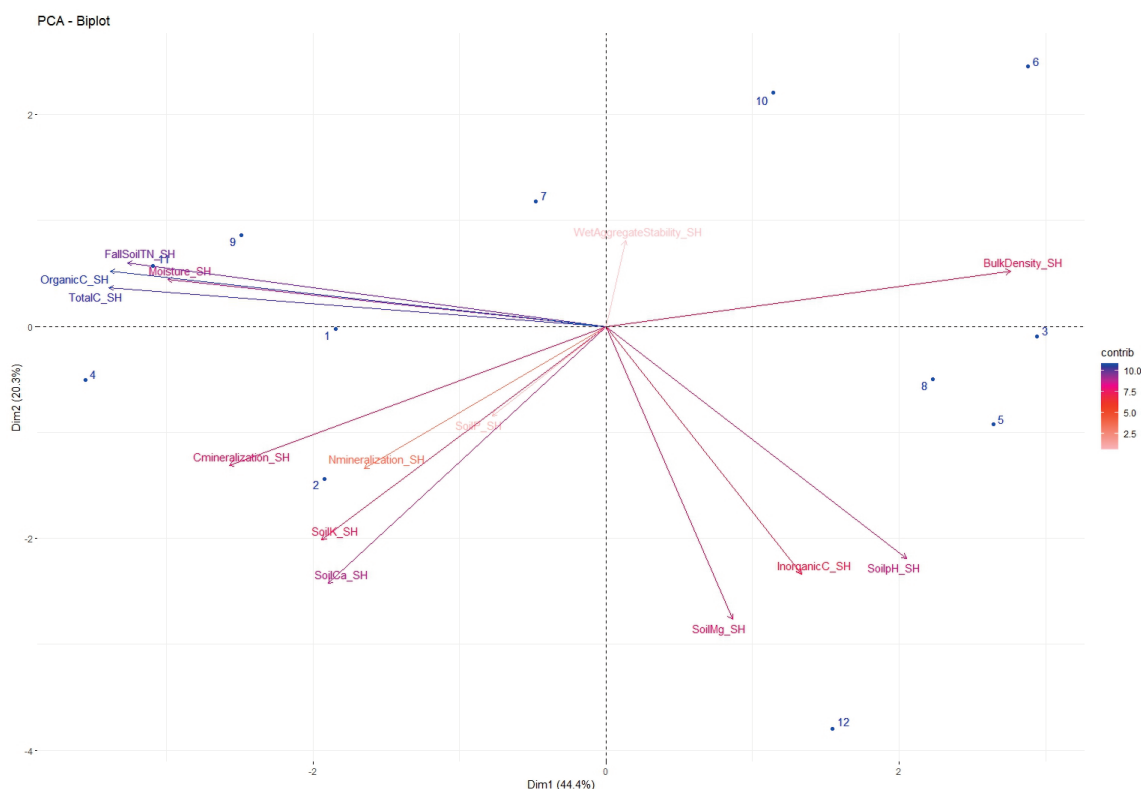


Figure 1. Principal component analysis for the relationship between soil health indicators and different crop practices across 20 years affected by treatments; 12 treatments are shown in dark blue dots in biplot. The contribution of soil health indicators is shown by different colors. Dark blue shows the highest contribution of organic C_SH, Total C_SH, followed by Fall Soil TN_SH (dark purple), and soil Ca, moisture, and PH in the sequential order (purple). Wet aggregate shows the least contributions (pink). The first principal component (PC1) and the second principal component (PC2) capture 44.4% and 20.3% of total variations among 12 treatments, respectively. Soil C, total C, Fall-Soil-TN, and moisture are positively related to each other but negatively related to bulk density, soil Mg, Inorganic C, and soil PH.

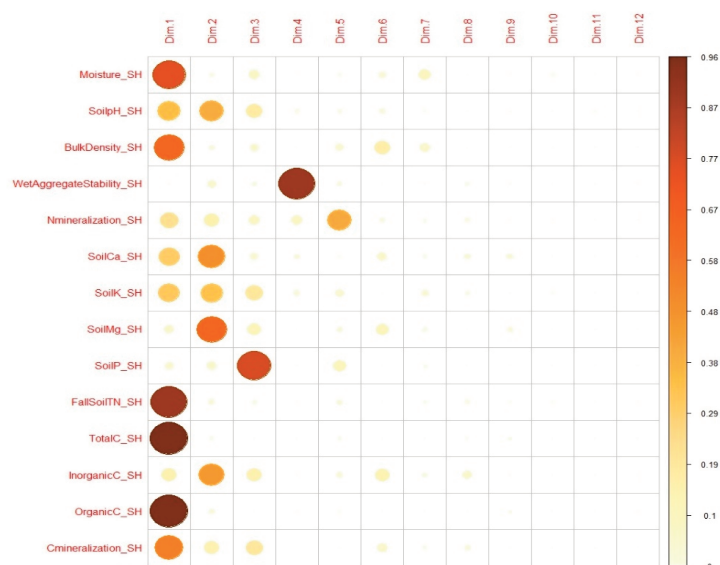


Figure 2. Principal component analysis for the contribution of all soil health indicators according to 12 principal components (Dims); Dim.1 includes organic carbon, total carbon, fall soil total nitrogen, and moisture. Dim. 2 includes soil Ca, Mg, and PH. Contribution of soil P and wet aggregate stability are shown in Dim.3 and Dim.4.

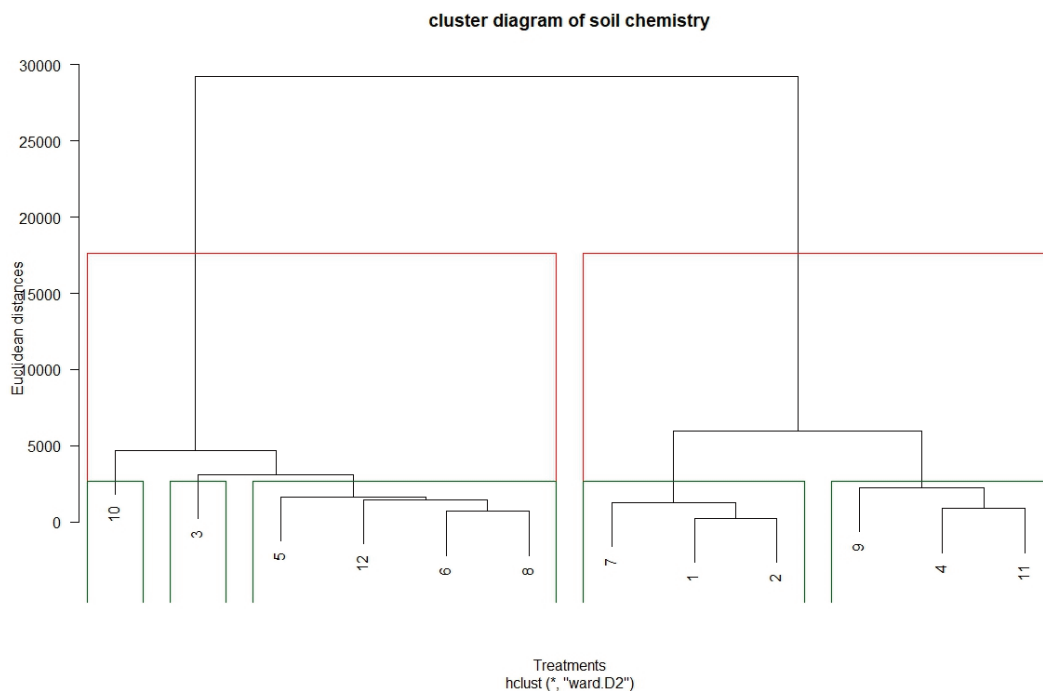


Figure 3. According to the dendrogram, Treatments 1 (perennial; Prairie) and 2 (perennial; Miscanthus) are the first two lines that merged, followed with treatments 4 (perennial; switchgrass) and 11 (corn with 0 fertilizer), then treatments 6 (corn/soybean with fertilizer) and 8 (corn+ rye grass with fertilizer) record the highest similarities in terms of soil health indicators compared to other practices in order. At the second stage, treatments 7, 9, and 12 joined the clusters mentioned above in order and shortly thereafter. Back on the left, although treatments 6, 8, 12, 5, and 3 are clustered and similar to each other, they differ more among themselves than to any other treatment in the data set.

PCA analysis showed that N fertilization had great impact on fall TN, but bulk density increased in annual crops. The highest rate of TC and organic carbon was recorded in treatments 1, 2, and 4, which are perennials (Figure 1). Wet aggregate had low contribution for total treatments compared to other soil indicators.

The highest pH was recorded in treatment 12, which is continuous corn (annual) treated with N fertilization for long time. Application of N fertilizer to corn and corn/soy in every other year may have maintained pH and bulk density.

The first principal component (PC1) of the PCA analysis captured 44% of the total variation between treatments. This component included carbon, total nitrogen, bulk density, and moisture, indicating their significant contributions to the observed variation (as illustrated in Figure 2). The second principal component (PC2) accounted for 20% of the total variation between treatments. It consisted of soil calcium (Ca), magnesium (Mg), and pH, suggesting their influence on the observed variation. To further analyze the relationships among the treatments, hierarchical clustering with Ward's minimum variance algorithm was employed. This clustering method creates a hierarchical decomposition of the dataset, forming a dendrogram—a tree-like structure that recursively splits the database into smaller subsets. The clustering helps identify similarities and dissimilarities between treatments based on their soil health indicators.

Euclidean distance was applied to measure similarity of the groups. The dendrogram shows two main big cluster around 6000 Euclidean distances. Fertilizer application had significant impact on clustering the treatments. No fertilizer was added to treatments 1, 2, 4, 7, 9, and 11 that are clustered separately from other treatments. Moreover, all three groups of perennial covers (Treatment 1, 2, 4) were grouped in this cluster. However, treatment 1 (Prairie) and 2 (Miscanthus) recorded the highest similarity with minimum Euclidean distances. Tillage effect was not significant in grouping treatments because half of treatments in each group were under no-tillage (treatments 1, 2, 4), and the rest of them were with conventional tillage (treatments 7, 9, 11). Fertilization was applied to treatments 10,

3, 5, 12, 6, and 8, which all of them were in the same cluster (Figure 3).

CONCLUSIONS

According to our results the most promising soil health indicator connected to different cropping practices were organic carbon, total carbon, fall soil total nitrogen, bulk density, soil Ca, moisture, and pH in the sequential order (Figure 1). Wet aggregate, soil P, and N mineralization showed less contribution, but organic carbon, total carbon, soil total nitrogen, and moisture explained 44% of the total variation (PC1). Moreover, Ca, Mg, and pH explained 20% of the total variation between treatments (PC2) (Figure 2).

The results of indicators comparison recorded that no-fertilization in combination to perennial covers caused the most promising distinction between treatments. Cover cropping such as white clover (treatment 5) and cereal rye side dress in corn (treatment 8) did not have any similarity to perennial cropping system (treatment 1, 2, 4). However, cereal rye side dress in soybean (treatment 9) recorded more similarity to switchgrass first and then to prairie and miscanthus (Figure 3).

This study indicates that perennial covers and cereal rye cultivated with soybean increase soil health and quality, where organic carbon, total carbon, and total nitrogen have highest contribution to these cropping practices in the most top soil layers. These data help us to choose an appropriate cropping system in future specially in climate change. Several studies have evaluated SOC changes under biofuel cropping systems compared to other land use systems (Kahle et al., 2001; Clifton-Brown et al., 2007). Although individual empirical experiments provide valuable insights, it is necessary to synthesize their results in order to expand the scope of inference across a wide range of environmental conditions in which bioenergy crops are expected to be cultivated. When biofuel crops such as perennial grasses are implemented as part of the landscape, there is a general expectation that carbon sequestration will increase, leading to a reduction in greenhouse gas emissions.

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